

PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Docket No: Q83408

Kousuke TANI, *et al.*

Appln. No.: 10/506,536

Group Art Unit: 1626

Confirmation No.: 1208

Examiner: Chung, Susannah Lee

Filed: September 03, 2004

For: 8-AZAPROSTAGLANDIN DERIVATIVE COMPOUNDS AND DRUGS
CONTAINING THE COMPOUNDS AS ACTIVE INGREDIENT

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Tsutomu Shiroya, hereby declare and state:

THAT I am a citizen of Japan;

THAT I have received the degree of a Master of Science in 1989 from Kyoto
Pharmaceutical University;

THAT I have been employed by Ono Pharmaceutical Co., Ltd. since 1989, where I
hold a position as HEAD of pharmacology Group 3, with responsibility for study of
prostaglandin at the Research Institute of Ono Pharmaceutical Co., Ltd.;

THAT this declaration is made for support of the above identified U.S. Patent
Application;

THAT I am a co-inventor of the present application; and

THAT the following experiments (Activity of EP₂ Receptor Agonist) were conducted
under my direct supervision.

EP₂ Receptor Binding Activity

Experiment for measurement of the an EP₂ receptor binding activity with the
cells expressing prostanoid receptor sub-types

According to the method of Sugimoto *et al.* (*J. Biol. Chem.*, 267, 6463-6466 (1992)), CHO cells which expressed prostanoid receptor sub-types (murine EP₁, EP₂, EP_{3α}, and EP₄, respectively) were prepared and used as membrane authentic samples.

A reaction solution (200 µl) containing the prepared membrane fraction (0.5 mg/ml) and ³H-PGE₂ was incubated at room temperature for 1 hour. The reaction was terminated with ice cold buffer (3 ml), and the reaction mixture was filtered under suction through a glass filter (GF/B), on which the binding ³H-PGE₂ was trapped. The binding radioactivity was measured by means of a liquid scintillator.

The K_d value was obtained from the Scatchard plots [*Ann. N.Y. Acad. Sci.*, 51, 660 (1949)]. Non-specific binding was obtained as the binding in the presence of an excess amount (2.5 µM) of unlabelled PGE₂. Measurement of the binding inhibition for ³H-PGE₂ with the compounds of the present invention was performed by adding ³H-PGE₂ (2.5 nM) and a series of concentrations of the compound of the present invention. In this reaction, the following buffer was used in all cases.

Buffer: 10 mM potassium phosphate (pH 6.0), 1 mM EDTA, 10 mM MgCl₂, and 0.1M NaCl.

Dissociation constant K_i (µM) of each compound was calculated from the following equation.

$$K_i = IC_{50} / (1 + ([C] / K_d))$$

IC₅₀ : The concentration of the compound of the present invention which inhibits half of the specific binding of [³H]PGE₂

C : The concentration of [³H]PGE₂

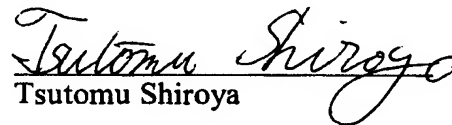
K_d : The dissociation constant of [³H]PGE₂

The results are shown below.

Example No.	K _i (nM)
	EP ₂
6(32)	0.5
6(48)	3.5
6(53)	1.0
6(60)	0.4
6(63)	0.4
6(74)	1.5
6(77)	0.4
6(89)	0.6

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: August 30, 2007


Tsutomu Shiroya